

Research article

Circulating soluble Fas levels and risk of ovarian cancer

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Abstract

Background: Dysregulation of apoptosis, specifically overexpression of soluble Fas (sFas), has been proposed to play a role in the development of ovarian cancer. The main objective of the present study was to evaluate serum sFas as a potential biomarker of ovarian cancer risk.

Methods: The association between serum sFas levels and the risk of ovarian cancer was examined in a case-control study nested within three prospective cohorts in New York (USA), Umeå (Sweden), and Milan (Italy). Case subjects were 138 women with primary invasive epithelial ovarian cancer diagnosed between 2 months and 13.2 years after the initial blood donation. Control subjects were 263 women who were free of cancer, and matched the case on cohort, menopausal status, age, and enrollment date. Serum sFas levels were determined using a quantitative sandwich enzyme immunoassay.

Results: Serum sFas levels were similar in women subsequently diagnosed with ovarian cancer (median, 6.5 ng/mL; range, 4.4 – 10.2) and in controls (median, 6.8 ng/mL; range, 4.5 – 10.1). Statistically significant trends of increasing serum sFas with age were observed among cases ($r = 0.39$, $p < 0.0001$) and controls ($r = 0.42$, $p < 0.0001$). Compared to women in the lowest third, women in the highest third of serum sFas were not at increased risk of ovarian cancer after adjustment for potential confounders (odd ratio (OR), 0.87; 95% confidence interval (CI), 0.42 – 1.82).

Conclusion: The results suggest that serum sFas may not be a suitable marker for identification of women at increased risk of ovarian cancer.

Background

Ovarian cancer remains the most deadly gynecologic tumor in the United States [1] and Europe [2]. In 2003, the American Cancer Society estimated that 25,400 women in the United States will be diagnosed with ovarian cancer, and 14,300 will die from the disease [1]. Accumulating experimental evidence suggests that the dysregulation of apoptosis, specifically a failure to eliminate ovarian epithelial inclusion cysts in the stroma, may play a role in the pathogenesis of the disease [3,4]. One of the major regulators of programmed cell death or apoptosis is the Fas/FasL system. Fas (Apo-1, CD95) is a member of the tumor necrosis factor receptor (TNF-R) superfamily [5] and can occur as both a cell-surface and a soluble protein (sFas). The interaction between Fas ligand (FasL) and cell-surface Fas induces apoptosis in sensitive cells through the activation of an intracellular Fas-associated death domain, the recruitment of caspase-8 and the activation of a cascade of proteases, resulting in proteolysis and degradation of chromosomal DNA [5]. Soluble Fas is usually generated by alternative mRNA splicing and lacks a transmembrane domain. By binding to FasL, sFas is thought to inhibit cell-surface Fas-FasL signaling and downregulate Fas-mediated apoptosis [6]. Elevated serum levels of sFas have been detected in ovarian cancer patients [4,7,8]. Serum sFas levels have been shown to correlate directly with advanced stage and increased tumor burden [9] and have been proposed as a prognostic factor in patients with gynecologic malignancies [8]. It has been hypothesized that circulating levels of sFas may be also reflective of risk of subsequent development of ovarian cancer. The objective of the present study was to evaluate the association between serum sFas levels and risk of ovarian cancer using samples collected in advance of clinical diagnosis from three collaborating prospective cohorts.

Methods

Study cohorts

The prospective cohorts in this study included the New York University Women's Health Study (NYUWHS), the Northern Sweden Health and Disease Study (NSHDS), and the Italian study of Hormones and Diet in the Etiology of Breast Tumors (ORDET). The cohorts have been described in detail previously [10-12]. Overall study coordination was provided through the International Agency for Research on Cancer (IARC).

At the time of recruitment, cohort members were administered questionnaire focusing on demographic (race, ethnicity, occupation), anthropometric (height, weight, body mass index), reproductive history (age at menarche, age at first full-term pregnancy, age at menopause), family history of cancer, medical history, and medication use. The quality of information collected at baseline was compara-

ble between the three participating cohorts. Subjects who reported hormone use at baseline were not eligible in the NYUWHS and the ORDET cohorts, and potential case and control subjects from the NSHDS who reported use of exogenous hormones were also considered ineligible for this study. At recruitment, all cohort members were asked to donate venous blood samples, which have been stored at -80°C for subsequent biochemical analyses. Samples chosen for this study were selected among the specimens that never undergo freeze-thaw cycles previously.

Nested case-control study

Eligible cases were subjects with primary, invasive epithelial ovarian cancer diagnosed at least 2 months after the initial blood donation and identified within each parent cohort by the date of the last complete follow-up. In the NYUWHS, follow-up consists of periodic (every 2-5 years) contacts of subjects by mail and telephone, as well as record linkages with the statewide tumor registries of New York, New Jersey, Connecticut and Florida, and with the U.S. National Death Index. In the NSHDS, ovarian cancer cases were identified through linkages with regional and national cancer registries and the vital status of the study participants was ascertained by record linkage to the regional and national registries for all-cause mortality. The ORDET database was linked to the regional Lombardy Cancer Registry to identify ovarian cancer cases, and to the regional residents' files to verify the vital status of the cohort members. The dates of last complete follow-up were January 1998, December 2000, and January 1997 for the NYUWHS, NSHDS, and ORDET, respectively. A total of 138 ovarian cancer cases met the eligibility criteria and were included in this study from the three parent cohorts (74 cases from the NYUWHS, 47 cases from the NSHDS, and 17 cases from the ORDET study). Additional serum samples collected during annual examinations were available for a subset of the NYUWHS subjects (30 cases, 59 controls).

For each case, two controls per case were randomly selected among appropriate risk sets. The risk set for a given case included cohort subjects who were alive and free of cancer, have not had a bilateral oophorectomy, and matched the case on cohort, age (± 6 months), date of recruitment (± 3 months), self-reported menopausal status at enrollment, and, for premenopausal women, day of the menstrual cycle at blood donation (for NYUWHS and ORDET subjects only). The goodness of matching for menopausal status was confirmed by FSH measurements (FSH levels ≤ 12.5 μ IU/mL were considered premenopausal), as previously described [13,14]. A total of 276 eligible matching controls were identified. Eleven potential controls were excluded because of the lack of serum and two controls were excluded because their FSH levels did not match their reported menopausal status. A total of

138 ovarian cancer cases and 263 matching controls were included in the study.

The Institutional Review Boards of the New York University School of Medicine, the University of Umeå and Istituto Nazionale Tumori in Milan reviewed and approved the present study.

Laboratory analyses

The samples from the NSHDS and ORDET were shipped on dry ice to the Reproductive Biology Research Laboratory at the Department of Obstetrics and Gynecology, NYU School of Medicine. Levels of sFas were measured at the NYU School of Medicine laboratory using a quantitative sandwich enzyme immunoassay technique according to manufacturer (Quantikine Human sFas Immunoassay, R&D Systems Inc., MN). Blood serum samples from each case and her matching controls were always analyzed in the same batch by a laboratory technician who was unaware of their disease status. Concentrations of sFas in nanograms per milliliter (ng/mL) were calculated using a standard curve generated for each set of samples. All samples were analyzed in duplicate and the average of two measurements was used to calculate serum sFas levels. The assay sensitivity defined as the minimum detectable concentration of sFas is 0.02 ng/mL. The intra-assay and inter-assay coefficients of variation provided by the laboratory were 3.1% and 4.5%, respectively. Furthermore, this assay demonstrated no significant cross-reactivity or interference with 92 common cytokines and growth factors.

Statistical analyses

In order to reduce departures from the normal distribution, sFas levels, weight, height, and body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters) were log-transformed. A mixed effects linear regression model was used to compare continuous variables between cases and controls, taking into account the matched design [15]. Categorical characteristics of cases and controls were compared using the conditional logistic regression model. Spearman correlation coefficients were calculated to quantify the strength of associations between serum sFas levels and various subjects' characteristics. A multivariate linear regression model estimated by the least squares was also used to identify factors predictive of sFas levels.

To compute odds ratios for ovarian cancer, sFas measurements were categorized into tertiles, using the frequency distribution of cases and controls combined. The conditional logistic regression model was used to take into account the matching. Odds ratios were computed relative to the lowest tertile. The effect of potential confounding variables (parity, use of oral contraceptives, and BMI) was assessed by including these variables in the logistic

regression model containing sFas one at a time, as well as by including them simultaneously. When assessing the effect of BMI, height was also included in the model because it has been shown that, if obesity is a risk factor, including only BMI in a model forces an inverse-quadratic relation of height to risk given weight [16]. Likelihood ratio tests were used to assess statistical significance and p-values for trends. Reported trend test p-values correspond to sFas treated as an ordered categorical variable. The statistical analyses were performed using Statistical Analysis Software, Version 8.0 (SAS Institute Inc., Cary, NC).

Results

Common epithelial-type ovarian tumors were observed, such as serous ($n = 59$), mucinous ($n = 14$), endometrioid ($n = 14$), clear cell ($n = 7$), mixed ($n = 1$), and not-otherwise-specified adenocarcinoma ($n = 25$). Histology was unknown for eighteen cases. For all cases combined, median period from the initial visit to the diagnosis of ovarian cancer was 5.1 years (range, 2 months – 13.2 years).

Selected descriptive characteristics of study subjects stratified by participating cohort are presented in Table 1 (see Additional file 1). The median age at diagnosis of ovarian cancer was 61 years in the NYUWHS, 59 years in the NSHDS study, and 54 years in the ORDET study. Because of the matching, ovarian cancer cases and controls had similar proportions of premenopausal and postmenopausal women and similar age at enrollment. There were no significant differences in the ages at menarche or at first full-term pregnancy between the cases and controls. Ovarian cancer cases were more likely to report nulliparity, later age at menopause (all cohorts), a positive first-degree history of breast cancer (the NYUWHS and the ORDET cohorts) and less likely to report use of oral contraceptives (the NSHDS and the ORDET cohorts) as presented in Table 1 (see Additional file 1). Among all subjects, significant case-control differences were observed in parity ($p = 0.02$) and age at menopause ($p = 0.03$). In agreement with previous report based on the same cohorts [14], in the combined studies analysis ovarian cancer cases had lower BMI (mean, 25.0 kg/m²), as compared to control subjects (mean, 26.0 kg/m²) ($p < 0.04$).

Table 1 reports the means, medians, standard deviations and ranges of serum sFas levels in ovarian cancer cases and controls. No statistically significant differences in sFas levels were observed between cases and controls in the total study population, within the participating cohorts, and after stratification by menopausal status. Serum sFas levels were slightly higher in the NSHDS subjects, as compared to the NYUWHS and ORDET subjects but the differences between cohorts were not statistically significant (Table 1).

Table 2: Serum sFas levels in ovarian cancer cases and controls from the three participating cohorts

Cohort	Serum sFas, ng/mL		
	Cases	Controls	P-value*
NYUWHS			
n	74	145	0.37
Mean (SD)	6.8 (2.2)	6.7 (2.1)	
Median (10th–90th percentiles)	6.5 (4.3 – 9.8)	6.5 (4.2 – 9.5)	
NSHDS			
n	47	86	0.20
Mean (SD)	8.1 (7.4)	8.0 (2.2)	
Median (10th–90th percentiles)	6.6 (4.8 – 10.9)	7.6 (5.5 – 11.2)	
ORDET			
n	17	32	0.07
Mean (SD)	6.2 (1.3)	6.7 (1.3)	
Median (10th–90th percentiles)	6.6 (3.9 – 7.6)	6.4 (5.2 – 8.1)	
Total			
n	138	263	0.51
Mean (SD)	7.2 (4.6)	7.1 (2.2)	
Median (10th–90th percentiles)	6.5 (4.4 – 10.2)	6.8 (4.5 – 10.1)	

* Mixed effect linear regression model (after natural log-transformation). SD, standard deviation.

Table 3: Odds ratios of ovarian cancer for tertiles of serum sFas levels

Serum sFas tertiles, ng/mL	Cases n (%)	Controls n (%)	OR (95%CI)	
			Unadjusted*	Adjusted**
<5.9	51 (37%)	83 (32%)	1.00	1.00
5.9 – 7.6	46 (33%)	88 (33%)	0.86 (0.50 – 1.48)	0.93 (0.49 – 1.78)
>7.6	41 (30%)	92 (35%)	0.69 (0.37 – 1.28)	0.87 (0.42 – 1.82)
p for trend***			0.24	0.71

* Except for matching on cohort, age, menopausal status, and date of blood donation. ** Adjusted for parity, use of oral contraceptives, BMI (log-transformed), and height (log-transformed). *** Conditional logistic regression model (sFas treated as ordered categorical variable).

The associations between serum sFas levels expressed as tertiles and risk of ovarian cancer are reported in Table 2. No statistically significant associations were observed in unadjusted analyses, nor in analyses adjusting for potential confounders (parity, use of oral contraceptives, BMI, and height) included in the statistical model either one at a time, or simultaneously. Both unadjusted and adjusted simultaneously for potential confounders, models are presented.

Because it has been suggested that sFas can be produced and shed into circulation by tumor [9], we explored the hypothesis that sFas levels increase closer to diagnosis with increasing ovarian tumor load and could serve as a marker of yet undiagnosed disease. First, we assessed serum sFas levels stratified by lagtime to the index date (ovarian cancer diagnosis). There were no significant dif-

ferences in sFas levels stratified by lagtime to the index date among ovarian cancer cases and their matching controls (Figure 1). We also observed no statistically significant associations between prediagnostic serum sFas and tumor stage ($P_{\text{trend}} = 0.54$) or grade ($P_{\text{trend}} = 0.76$).

In addition, we examined the changes over time in sFas levels among subjects for whom repeated blood donations were available (30 cases and 59 matched controls from the NYUWHS cohort). For cases, median time between the first and second visits was 376 days, median sFas difference between the first and repeated measurements was -0.19 ng/mL and median rate of change was -4.1×10^{-4} ng/mL/day. For controls, median time between two visits was 378 days, median sFas difference between the first and repeated measurements was 0.10 ng/mL and median rate of change was 2.8×10^{-4} ng/mL/day. The

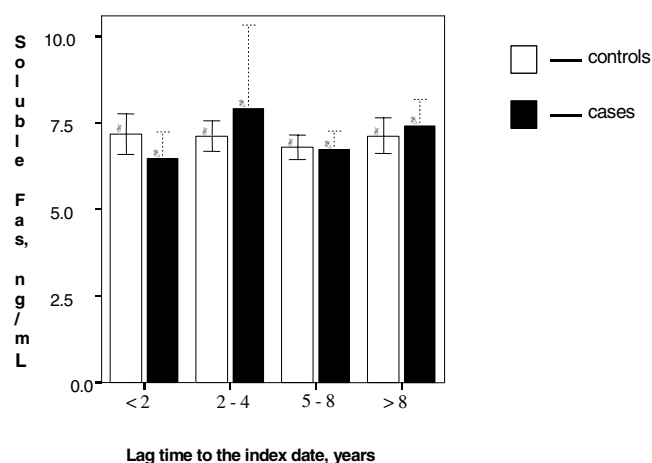


Figure 1

Levels of sFas by lagtime from the blood donation to the index date (ovarian cancer diagnosis). Bars show means and 95% confidence intervals. There were no significant differences in sFas levels stratified by lagtime to the index date among ovarian cancer cases ($p = 0.38$) and matching controls ($p = 0.80$).

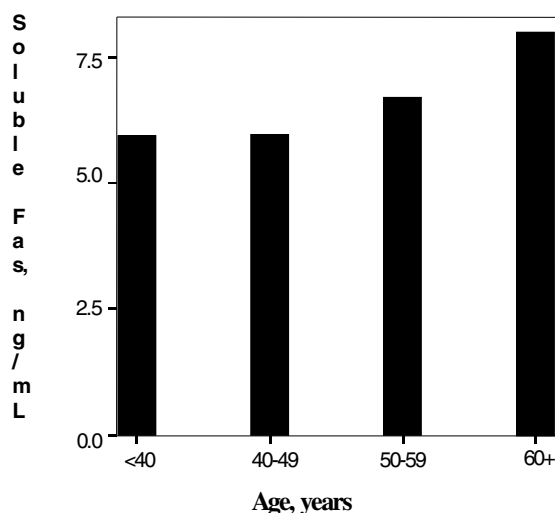


Figure 2

Correlation between age and sFas levels in the control group. There was a significant correlation (Spearman $r = 0.42$, $p < 0.0001$) between age and sFas (median levels presented).

difference in rate of change between ovarian cancer cases and matched controls with repeated blood donations was

not statistically significant ($p = 0.81$). Spearman correlation coefficient between first and second sFas measurements was 0.72 ($p < 0.0001$).

Little is known about factors that determine physiological sFas levels. To identify the factors that may be related to serum sFas, we examined correlations of sFas levels with known characteristics of control subjects. There was a direct and statistically significant correlation between serum sFas and age (Spearman $r = 0.42$, $p < 0.0001$) (Figure 2). Similar positive correlation between serum sFas and age was observed among cases (Spearman $r = 0.39$, $p < 0.0001$). We also analyzed the effect of menopausal status on sFas levels. Results of a linear regression analysis indicated that sFas levels were positively associated with age ($p < 0.002$) but not with menopausal status ($p = 0.52$). After adjustment for age, no significant correlations were observed between serum sFas and selected subjects' characteristics, such as age at menarche, age at first full-term pregnancy, age at menopause, weight, height, and BMI.

Discussion

The Fas-FasL system is a primary mediator of apoptosis in many major physiologic processes. The proposed roles of Fas-mediated apoptosis include the elimination of activated T-cells following an immune response, the killing of tumor and virally infected cells by cytotoxic T-cells and natural killer cells, and maintenance of immune-privileged sites such as the eye, testis, and maternal-fetal interface.

Dysregulation of Fas-mediated apoptosis is thought to play a role in the development of various tumors, including ovarian cancer [3,4]. It has been proposed that cancer cells expressing FasL have an advantage to evade human immune surveillance by inducing apoptosis in infiltrating lymphocytes expressing Fas. It is known that some tumor cells express both Fas and FasL yet are resistant to Fas-mediated apoptosis. This phenomenon has been attributed to receptor dysfunction, or intracellular inhibition by FLICE inhibitory protein (FLIP) that blocks Fas-mediated tumor cell death, enabling the tumor to avoid immune clearance [17].

Soluble form of Fas receptor, which is lacking the transmembrane domain of the cell-surface Fas, is thought to compete for FasL with Fas receptor expressed on tumor cells and, therefore, alter or prevent apoptotic signal transduction [6]. Previously, several hospital-based case-control studies have shown that circulating sFas levels were significantly elevated in ovarian cancer patients as compared to controls [4,7,8] and it has been proposed that serum sFas levels or FasL expression may serve as a prognostic factor in patients with ovarian cancer and other gynecological malignancies [8,18].

The objective of the current study was to evaluate serum sFas as a predictive marker of ovarian cancer risk. In this case-control study nested within prospective cohorts, we observed no association between serum sFas levels and subsequent risk of subsequent ovarian cancer, before and after adjustment for potential confounders.

Several factors should be taken into consideration in the interpretation of these results. The levels of serum sFas observed in our study were markedly higher than in earlier studies [4,7,8]. While two of the previous studies [7,8] used sFas ELISA assays (Medical & Biological Laboratories Co., Nagoya, Japan), the present study used a quantitative sandwich enzyme immunoassay technique (Quantikine Human sFas Immunoassay, R&D Systems Inc., MN), a very sensitive and specific assay. Therefore, the observed differences in serum sFas levels between the current and previous studies could reflect variations in laboratory assay methods.

The discrepancy in results between this case-control study nested within prospective cohorts and previous retrospective hospital-based studies may also be related to the origins of sFas. Although the precise cellular source of serum sFas remains unclear, increased sFas levels in culture supernatants of breast and colon cancer cells lead to the assumption that sFas is derived from tumor cells [9]. It has been shown that serum sFas are elevated in patients with more advanced tumor stages [7-9] and that serum sFas levels decrease after surgical resection, as compared with levels obtained before surgery [9]. These findings suggest that sFas is produced and shed into circulation by tumor and that serum sFas levels could be reflective of tumor bulk. This may explain the results of previous hospital-based case-control studies suggesting that sFas can be used as a prognostic marker in patients with advanced ovarian cancer, as well as the lack of association between serum sFas and ovarian cancer observed in this prospective study, for which serum samples were collected up to 13.2 years before diagnosis.

Soluble Fas can also be produced by activated peripheral blood lymphocytes [19] and it has been postulated that dysfunction of apoptotic pathways or production of soluble factors including sFas and soluble FasL may be involved in the pathogenesis of several autoimmune diseases [20] and in cancer [3,4,21]. We observed a strong positive correlation between age and serum sFas suggesting that circulating sFas levels are increasing with age. Because the incidence of cancer and autoimmune disease also increases with age, this observation provides additional indirect evidence for a possible role of apoptosis dysregulation in these conditions. Future studies of sFas should consider taking into account the age-related changes in circulating sFas levels.

Conclusions

In conclusion, previous studies have demonstrated that serum sFas could be a useful prognostic factor in women who are already diagnosed with ovarian cancer. However, the results of the present study, which observed lack of association between prediagnostic serum sFas and ovarian cancer diagnosed later in life, suggest that serum sFas may not be a suitable susceptibility marker for identification of women at increased risk of the disease.

List of abbreviations

BMI, body mass index; CI, confidence interval; ELISA, enzyme-linked immunoassay; FasL, Fas ligand; FLIP, FLICE inhibitory protein; FSH, follicle-stimulating hormone; IARC, International Agency for Research on Cancer; ng/mL, nanograms per milliliter; NSHDS, Northern Sweden Health and Disease Study; NYUWHS, New York University Women's Health Study; ORDET, Hormones and Diet in the Etiology of Breast Tumors study; OR, odds ratio; sFas, soluble Fas receptor; TNF-R, tumor necrosis factor receptor.

Competing interests

None declared.

Authors' contributions

AA, AL, PT participated in study design and preparation of the manuscript. AA and YA participated in sample preparation from the NYUWHS site. EL and PL participated in sample preparation from the NSHDS site. AM and VK participated in sample preparation from the ORDET site. SR participated in sample preparation and aliquoting at the IARC. SG and YM performed the laboratory analyses. YA and AZ-J participated in the statistical analyses. AA, PM, RK, FB, GH and PT participated in the study coordination, manuscript editing and final manuscript preparation.

Additional material

Additional File 1

Characteristics of ovarian cancer cases and controls in the three participating cohorts

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